EXHIBIT A, PAGE 1

164 AN

→ Sacrifice de souris : effet adjuvant de chemokines (hMCP-4, hMIP-3α, hTECK peprotech) sur la réponse α βGal (plasmide) immunisations: injection de 50 μl de PBS ou 50 μl de chemokine dans PBS (=100ng), et 3h après 50 μg (50 μl) en footpad de placZ ou pCDNA3, endotoxin negligeable (< 5EU)
Injections identiques à J0, J7, J14, J21. Prises de sang pour sérum à J0, J14, J28.
J28: sacrifice de toutes les souris (groupes de 6 ♀ C57BL/6, 6s en début de protocole). Prélèvement rate + ganglions poplités.
Mise en suspension, déplétion G.R. pour rates.
numération

	ganglion poplité		rate	
	total × 10⁻⁶	/animal × 10⁻⁶	total × 10⁻⁶	/animal × 10⁻⁶
pcDNA3	9.66	~~1.93~~ 1.61	265	44.17
38 + placZ	18.96	~~3.79~~ 3.16	285	47.50
IP-3α + placZ	21.6	~~4.32~~ 3.60	250	41.67
CP-4 + placZ	33.0	~~6.60~~ 5.50	325	54.17
ECK + placZ	22.8	3.80	300	50.00

Mise en culture en milieu clone + 20ug IL-2 + 1μM BP-1
avec /ml: 5×10⁶ splenocytes (24 puits / groupe) ou 2×10⁶ cellules de ganglion (toutes les cellules mises en culture).
à J3: rajouter 1ml de milieu + IL2 + BP-1 .

EXHIBIT A, PAGE 2

AV 165

Vendredi

Mesure tumeurs inj. 6Ckine (mm²)

mGCK 1000 ng	m6CK 100 ng	m6CK 10 ng	PBS	h6CK 1000 ng	h6CK 100 ng	h6CK 10 ng
201.06	254.47	0	240.53	—	0	0
	14.34	201.06			165.13	78.53
		254.47			70.88	213.82
		165.13				

Lundi

Mesure tumeurs 6Ckine (mm²)

m6CK 1000 ng	m6CK 100 ng	m6CK 10 ng	PBS	h6CK 1000 ng	h6CK 100 ng	h6CK 10 ng
201.06	188.69	0	254.47	—	0	143.14
	254.47	254.47			78.53	86.59
		213.82				201.06
		153.93				

→ ELISA pour effet adjuvant hMCP-4, hMIP-3α (pas dosé TECK car pas assez de plaques coatées) cf p164 : groupes.
protocole dosage IgG, IgG1, IgG2a classique :

- coat 5 µg/ml, 50 µl in PBS O/N @ 4°C
- 2 lavages PBS.
- bloquer 2h @37°C
- échantillons poolés, 1/40° puis 1/2 en 1/2, triplicates. 1h @ RT
- α Igs 1/1000° SAL PAL 1h
- substrat 30'
- OD 405-480.

PAGE 15/20 * RCVD AT 8/13/2004 3:33:19 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/7 * DNIS:8729306 * CSID:908 298 5405 * DURATION (mm-ss):05-42

EXHIBIT A, PAGE 3

166

A1

Ig6 J0

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	0.019	0.002	10.01	0.018	0.020	0.019
A2	1	0.007	0.001	8.45	0.008	0.006	0.006
A3	1	0.001	0.001	200.00	-0.000	0.001	0.002
A4	1	-0.000	0.001	208.00	-0.001	0.001	-0.000
A5	1	-0.001	0.001	49.49	-0.001	-0.000	-0.001
A6	1	-0.002	0.003	105.83	-0.001	-0.000	-0.005
A7	1	-0.005	0.001	11.95	-0.005	-0.004	-0.004
B1	1	0.020	0.001	2.91	0.020	0.020	0.020
B2	1	0.008	0.002	19.50	0.010	0.006	0.008
B3	1	0.004	0.002	49.49	0.004	0.002	0.004
B4	1	0.002	0.001	26.65	0.002	0.002	0.002
B5	1	0.000	0.002	1249.99	-0.000	0.002	-0.001
B6	1	-0.001	0.003	215.71	-0.003	0.002	-0.002
B7	1	-0.004	0.001	13.86	-0.003	-0.004	-0.004
C1	1	0.038	0.004	10.67	0.038	0.034	0.043
C2	1	0.018	0.001	5.41	0.020	0.018	0.018
C3	1	0.010	0.003	28.57	0.008	0.010	0.014
C4	1	0.006	0.001	16.38	0.006	0.006	0.006
C5	1	0.002	0.001	40.00	0.002	0.002	0.004
C6	1	-0.001	0.005	507.10	-0.004	-0.002	0.004
C7	1	-0.001	0.002	157.95	-0.002	-0.002	0.000
D1	1	0.044	0.004	8.29	0.046	0.044	0.040
D2	1	0.025	0.002	8.43	0.026	0.024	
D2A3	1	0.027	-	-	0.027		
D3	1	0.014	0.005	33.94	0.018	0.012	0.010
D4	1	0.011	0.005	45.07	0.016	0.010	0.006
D5	1	0.004	0.003	98.97	0.008	0.002	0.002
D6	1	0.002	0.002	83.32	0.004	0.001	0.002
D7	1	-0.000	0.004	721.11	0.002	-0.004	0.001

Ig61 J0

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	-0.001	0.003	274.54	-0.001	0.002	-0.003
A2	1	-0.004	0.001	14.74	-0.004	-0.003	-0.004
A3	1	-0.004	0.001	33.22	-0.004	-0.004	-0.002
A4	1	-0.004	0.001	14.74	-0.004	-0.003	-0.004
A5	1	-0.004	0.001	29.40	-0.003	-0.005	-0.003
A6	1	-0.004	0.001	14.74	-0.003	-0.004	-0.004
A7	1	-0.006	0.000	0.03	-0.006	-0.006	-0.006
B1	1	-0.003	0.001	39.53	-0.004	-0.002	-0.002
B2	1	-0.004	0.002	47.06	-0.004	-0.006	-0.002
B3	1	-0.002	0.002	76.90	-0.001	-0.004	-0.001
B4	1	-0.002	0.001	60.25	-0.003	-0.001	-0.001
B5	1	-0.003	0.002	75.90	-0.008	-0.003	-0.003
B6	1	-0.003	0.002	59.73	-0.004	-0.002	-0.001
B7	1	-0.005	0.001	12.60	-0.004	-0.004	-0.005
C1	1	0.022	0.003	13.93	0.023	0.015	0.025
C2	1	0.013	0.002	18.78	0.015	0.009	0.022
C3	1	0.005	0.003	53.29	0.004	0.004	0.009
C4	1	0.002	0.001	37.71	0.005	0.002	0.002
C5	1	-0.000	0.002	672.62	-0.001	-0.001	0.002
C6	1	-0.001	0.006	544.15	-0.004	-0.003	0.006
C7	1	-0.000	0.005	1385.64	-0.002	-0.002	0.004
D1	1	0.010	0.011	108.06	0.020	0.011	-0.001
D2	1	0.010	0.003	29.01	0.008	0.012	
D2A3	1	-0.002	-	-	-0.002		
D3	1	0.000	0.008	9165.17	0.007	0.002	-0.008
D4	1	0.004	0.008	175.87	0.011	0.007	-0.004
D5	1	-0.001	0.008	603.99	0.006	-0.000	-0.009
D6	1	-0.001	0.005	879.30	0.004	0.001	-0.006
D7	1	-0.003	0.004	146.95	0.002	-0.004	-0.005

Ig62a J0

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	-0.005	0.004	68.68	-0.008	-0.001	-0.006
A2	1	-0.007	0.001	13.79	-0.007	-0.006	-0.008
A3	1	-0.007	0.002	23.70	-0.008	-0.006	-0.005
A4	1	-0.005	0.001	19.49	-0.004	-0.004	-0.006
A5	1	-0.006	0.001	9.76	-0.006	-0.005	-0.006
A6	1	-0.006	0.002	25.82	-0.006	-0.004	-0.007
A7	1	-0.008	0.002	19.30	-0.009	-0.008	-0.006
B1	1	-0.005	0.002	42.34	-0.007	-0.004	-0.003
B2	1	-0.007	0.003	36.49	-0.006	-0.010	-0.005
B3	1	-0.005	0.002	42.34	-0.004	-0.007	-0.003
B4	1	-0.005	0.002	45.62	-0.006	-0.003	-0.002
B5	1	-0.005	0.001	11.74	-0.005	-0.005	-0.004
B6	1	-0.003	0.003	79.18	-0.004	-0.004	-0.000
B7	1	-0.006	0.003	42.43	-0.003	-0.006	-0.008
C1	1	-0.002	0.003	192.95	-0.003	-0.004	0.002
C2	1	-0.001	0.004	440.89	0.004	-0.003	-0.003
C3	1	-0.002	0.003	117.58	-0.004	-0.003	0.001
C4	1	-0.001	0.001	82.38	-0.001	-0.000	-0.001
C5	1	-0.002	0.001	36.48	-0.002	-0.001	-0.001
C6	1	-0.003	0.007	215.36	-0.008	-0.006	0.005
C7	1	-0.003	0.005	175.34	-0.007	-0.004	0.003
D1	1	0.006	0.009	116.62	0.014	0.005	-0.000
D2	1	0.005	0.004	67.34	0.008	0.001	
D2A3	1	0.011	-	-	0.011		
D3	1	-0.001	0.007	734.68	0.006	-0.002	-0.007
D4	1	0.006	0.006	95.22	0.012	0.008	-0.000
D5	1	-0.001	0.005	515.54	0.003	0.001	-0.006
D6	1	-0.002	0.002	156.86	0.002	-0.001	-0.002
D7	1	-0.003	0.003	98.97	-0.001	-0.006	-0.001

Ig6 J14

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	0.027	0.002	6.34	0.028	0.028	0.025
A2	1	0.011	0.003	23.59	0.013	0.010	0.008
A3	1	0.004	0.001	19.75	0.003	0.003	0.004
A4	1	0.001	0.001	86.60	0.000	0.001	0.000
A5	1	-0.000	0.001	346.41	0.000	0.000	-0.002
A6	1	-0.002	0.001	37.74	-0.001	-0.003	-0.003
A7	1	-0.006	0.000	0.05	-0.006	-0.006	-0.006
B1	1	0.120	0.010	8.43	0.129	0.122	0.109
B2	1	0.055	0.003	5.63	0.058	0.052	0.055
B3	1	0.027	0.002	7.32	0.029	0.025	0.027
B4	1	0.013	0.001	8.88	0.014	0.012	0.012
B5	1	0.005	0.001	18.75	0.006	0.004	0.005
B6	1	0.002	0.003	113.39	-0.001	0.003	0.004
B7	1	-0.003	0.001	19.24	-0.003	-0.003	-0.004
C1	1	0.052	0.007	13.09	0.054	0.044	0.057
C2	1	0.028	0.001	2.35	0.026	0.025	0.035
C3	1	0.015	0.004	26.94	0.011	0.014	0.019
C4	1	0.007	0.001	13.64	0.007	0.008	0.006
C5	1	0.002	0.001	69.28	0.000	0.002	0.002
C6	1	0.001	0.004	606.22	-0.004	0.001	0.004
C7	1	-0.000	0.004	1053.57	-0.004	-0.001	0.003
D1	1	0.044	0.005	11.44	0.049	0.039	0.043
D2	1	0.028	0.003	9.98	0.026	0.030	
D2A3	1	0.017	-	-	0.017		
D3	1	0.013	0.005	37.31	0.016	0.014	0.009
D4	1	0.012	0.007	57.07	0.017	0.015	0.004
D5	1	0.003	0.005	157.40	0.008	0.002	-0.001
D6	1	0.003	0.002	86.60	0.005	0.002	0.001
D7	1	0.000	0.003	2078.96e+06	0.003	-0.004	0.002

Ig61 J14

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	0.002	0.002	120.49	0.001	0.005	0.001
A2	1	-0.003	0.001	30.59	-0.002	-0.003	-0.002
A3	1	-0.002	0.002	75.32	-0.003	-0.002	-0.000
A4	1	-0.003	0.002	55.55	-0.004	-0.003	-0.001
A5	1	-0.003	0.001	20.39	-0.003	-0.002	-0.002
A6	1	-0.004	0.001	22.64	-0.004	-0.003	-0.005
A7	1	-0.006	0.001	10.04	-0.006	-0.006	-0.005
B1	1	0.128	0.001	0.90	0.130	0.128	0.128
B2	1	0.071	0.004	6.10	0.076	0.069	0.068
B3	1	0.035	0.003	8.19	0.037	0.034	0.034
B4	1	0.012	0.000	9.04	0.019	0.013	0.019
B5	1	0.010	0.002	20.87	0.010	0.012	0.008
B6	1	0.003	0.001	17.76	0.004	0.004	0.003
B7	1	-0.001	0.002	141.08	-0.003	-0.001	0.001
C1	1	0.027	0.003	11.94	0.026	0.023	0.031
C2	1	0.013	0.001	6.94	0.014	0.014	0.013
C3	1	0.009	0.003	30.82	0.007	0.008	0.013
C4	1	0.006	0.002	25.82	0.006	0.008	0.005
C5	1	0.000	0.002	511.01	0.001	-0.001	0.002
C6	1	0.002	0.004	252.63	-0.002	0.002	0.006
C7	1	-0.000	0.002	1333.04	-0.001	-0.000	0.002
D1	1	0.012	0.006	52.48	0.020	0.010	0.008
D2	1	0.006	0.002	35.10	0.008	0.007	0.004
D3	1	0.003	0.005	193.55	0.008	0.003	-0.002
D4	1	0.007	0.006	84.29	0.013	0.009	0.001
D5	1	0.000	0.004	1103.79	0.005	0.001	-0.004
D6	1	0.001	0.003	344.80	0.005	0.001	-0.001
D7	1	-0.002	0.004	189.20	0.001	-0.006	-0.001

Ig62a J14

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	0.001	0.003	277.56	0.001	0.002	-0.003
A2	1	-0.004	0.002	53.15	-0.002	-0.005	-0.006
A3	1	-0.005	0.002	31.02	-0.006	-0.006	-0.004
A4	1	-0.006	0.001	9.24	-0.006	-0.007	-0.007
A5	1	-0.005	0.002	37.73	-0.004	-0.004	-0.007
A6	1	-0.005	0.000	8.00	-0.005	-0.005	-0.005
A7	1	-0.006	0.001	17.91	-0.007	-0.005	-0.006
B1	1	0.027	0.003	9.43	0.025	0.026	0.030
B2	1	0.009	0.003	28.10	0.011	0.006	0.010
B3	1	0.004	0.002	32.38	0.004	0.003	0.006
B4	1	0.002	0.002	87.29	0.001	0.000	0.003
B5	1	-0.001	0.003	203.35	0.001	-0.002	-0.004
B6	1	0.000	0.001	692.81	0.000	-0.001	0.000
B7	1	-0.004	0.002	35.94	-0.005	-0.006	-0.003
C1	1	0.038	0.005	13.53	0.043	0.032	0.039
C2	1	0.018	0.001	3.75	0.017	0.018	0.017
C3	1	0.008	0.004	43.45	0.004	0.008	0.012
C4	1	0.003	0.001	18.72	0.003	0.003	0.007
C5	1	-0.001	0.002	166.64	-0.003	-0.001	0.000
C6	1	-0.001	0.004	440.89	-0.005	-0.003	0.003
C7	1	0.004	0.006	141.40	0.009	-0.003	0.006
D1	1	0.023	0.006	23.98	0.030	0.020	0.019
D2	1	0.012	0.004	34.46	0.015	0.019	0.007
D3	1	0.004	0.006	124.06	0.010	0.003	-0.001
D4	1	0.008	0.004	42.84	0.012	0.007	0.005
D5	1	0.001	0.003	266.47	0.004	-0.001	-0.001
D6	1	0.001	0.002	277.56	0.002	-0.002	0.001
D7	1	-0.001	0.008	685.73	0.003	-0.009	-0.003

A: pcDNA3   C: hNIP-3α + placZ

B: placZ (+PBS)   D: hNCA4 + placZ

A1, 2, 3: dilutions 1/40, 1/80, 1/160...

EXHIBIT A, PAGE 4

16;

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	0.132	0.001	1.91	0.133	0.134	0.129
A2	1	0.059	0.001	1.72	0.060	0.059	0.057
A3	1	0.027	0.000	0.00	0.027	0.027	0.027
A4	1	0.011	0.001	9.18	0.010	0.011	0.012
A5	1	0.005	0.001	12.68	0.004	0.005	0.005
A6	1	-0.002	0.001	92.31	-0.001	-0.003	-0.002
A7	1	-0.003	0.001	32.43	-0.004	-0.003	-0.002
A8	1	0.941	0.029	3.14	0.974	0.951	0.917
A9	1	0.798	0.039	4.94	0.818	0.765	0.816
A10	1	0.574	0.045	7.46	0.596	0.525	0.602
A11	1	0.337	0.025	7.18	0.348	0.328	0.335
A12	1	0.203	0.022	11.65	0.208	0.181	0.218
B1	1	0.169	0.014	13.99	0.152	0.094	0.152
B2	1	0.053	0.007	13.22	0.052	0.047	0.061
B3	1	0.948	0.011	1.20	0.957	0.951	0.935
B4	1	0.810	0.009	1.11	0.820	0.804	0.803
B5	1	0.596	0.010	1.68	0.596	0.596	0.606
B6	1	0.402	0.001	0.28	0.401	0.403	0.403
B7	1	0.218	0.004	1.84	0.223	0.216	0.214
B8	1	0.133	0.005	4.00	0.131	0.138	0.128
B9	1	0.062	0.005	8.17	0.057	0.061	0.067
B10	1	2.053	0.005	0.23	2.048	2.054	2.057
B11	1	1.814	0.034	1.84	1.846	1.823	1.780
B12	1	1.419	0.007	0.47	1.421	1.425	1.411
C1	1	0.968	0.033	3.47	0.996	0.955	0.930
C2	1	0.536	0.017	3.24	0.546	0.533	0.512
C3	1	0.298	0.007	2.39	0.306	0.292	0.300
C4	1	0.157	0.004	2.89	0.161	0.157	0.153

Rg6
328

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	0.137	0.008	5.86	0.129	0.144	0.140
A2	1	0.066	0.004	5.36	0.069	0.067	0.062
A3	1	0.031	0.001	3.87	0.032	0.031	0.031
A4	1	0.013	0.002	11.83	0.014	0.015	0.012
A5	1	0.006	0.001	18.48	0.006	0.008	0.006
A6	1	0.001	0.001	93.38	0.001	0.001	0.001
A7	1	-0.003	0.001	38.72	-0.001	-0.003	-0.002
A8	1	0.468	0.032	7.14	0.476	0.415	0.448
A9	1	0.268	0.011	4.26	0.272	0.250	0.264
A10	1	0.145	0.008	5.56	0.145	0.138	0.140
A11	1	0.074	0.002	2.90	0.077	0.076	0.073
A12	1	0.037	0.002	5.47	0.039	0.037	0.035
B1	1	0.019	0.001	6.00	0.019	0.021	0.018
B2	1	0.006	0.002	24.44	0.008	0.007	0.005
B3	1	0.458	0.009	1.91	0.464	0.458	0.454
B4	1	0.265	0.009	3.27	0.275	0.260	0.260
B5	1	0.139	0.005	3.69	0.136	0.141	0.144
B6	1	0.076	0.003	4.23	0.080	0.073	0.074
B7	1	0.039	0.002	3.89	0.038	0.041	0.040
B8	1	0.020	0.004	20.29	0.018	0.016	0.025
B9	1	0.010	0.003	26.13	0.008	0.008	0.014
B10	1	2.969	0.069	2.42	2.972	2.904	2.972
B11	1	2.313	0.006	0.27	2.320	2.319	2.322
B12	1	1.569	0.040	2.54	1.533	1.590	1.584
C1	1	0.958	0.041	4.31	0.985	0.982	0.911
C2	1	0.563	0.013	2.27	0.571	0.558	0.553
C3	1	0.314	0.017	5.42	0.331	0.297	0.315
C4	1	0.165	0.016	9.55	0.180	0.158	0.154

Rg61
328

Sample	Dilution	Mean OD	SD OD	% CV OD	OD		
A1	1	0.043	0.003	6.18	0.041	0.046	0.041
A2	1	0.017	0.000	0.00	0.017	0.017	0.017
A3	1	0.007	0.002	31.51	0.006	0.009	0.007
A4	1	0.060	0.113	172.33	0.196	0.001	-0.000
A5	1	0.032	0.048	178.54	0.068	0.003	-0.003
A6	1	0.009	0.032	249.17	0.038	-0.004	-0.003
A7	1	-0.001	0.010	1983.99	0.011	-0.008	-0.004
A8	1	2.968	0.040	1.34	2.953	2.983	3.010
A9	1	2.549	0.091	3.58	2.586	2.445	2.616
A10	1	2.075	0.078	3.75	2.153	2.056	2.019
A11	1	1.398	0.056	3.55	1.437	1.414	1.343
A12	1	0.839	0.061	7.17	0.903	0.869	0.745
B1	1	0.478	0.038	7.90	0.508	0.488	0.434
B2	1	0.241	0.024	9.89	0.266	0.239	0.218
B3	1	2.948	0.050	1.71	2.953	2.975	2.916
B4	1	2.734	0.104	3.80	2.662	2.807	2.753
B5	1	2.198	0.077	3.51	2.189	2.247	2.228
B6	1	1.513	0.053	3.51	1.468	1.536	1.535
B7	1	0.930	0.067	8.79	0.916	0.923	0.952
B8	1	0.552	0.018	3.30	0.536	0.551	0.551
B9	1	0.245	0.029	11.84	0.246	0.251	0.239
B10	1	2.998	0.027	0.89	2.968	3.016	3.012
B11	1	2.882	0.049	1.69	2.844	2.927	2.976
B12	1	2.421	0.047	1.94	2.465	2.474	2.384
C1	1	1.756	0.104	5.92	1.835	1.790	1.637
C2	1	1.094	0.046	4.21	1.139	1.098	1.047
C3	1	0.628	0.013	2.10	0.641	0.615	0.632
C4	1	0.324	0.015	4.61	0.342	0.312	0.325

Rg62a
328

EXHIBIT A, PAGE 5

168